Near future or still a dream?
Non-invasive prenatal testing for monogenic diseases

TORO DELGADO, Nerea
Tutor: Vicenç Català Cahís

1 Introduction

What is cell-free fetal DNA?

The cell-free fetal DNA (cffDNA) is composed of small fragments of extracellular DNA derived from the shedding of placental trophoblasts that goes under apoptosis. This cffDNA fragments have a very short half-life and just represents the 10% of the cell-free DNA detected on maternal plasma. Despite of this small fraction, the whole fetal genome is represented in the cffDNA that can be found in the maternal bloodstream.
Since this discovery, many applications for cffDNA in non-invasive prenatal testing (NIPT) have been developed [Figure 1].

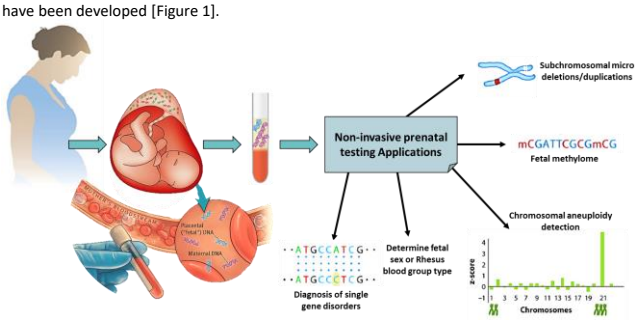

Figure 1. An overview of current non-invasive prenatal testing (NIPT) applications. Cell-free fetal DNA (cffDNA) proceed from apoptotic trophoblast and cross the placenta for arrive to the mother's blood stream. This cffDNA can be collected with a blood sample and after its extraction, cffDNA can be used for NIPT. Many NIPT applications for cffDNA have been developed through the years: establishing fetal sex or the Rhesus blood group type, screening for fetal aneuploidies, screening for microdeletions and microduplications syndromes, diagnosis of monogenic disorders and also the fetal methylome and transcriptome have been investigated.

Why monogenic diseases?

The application for NIPT in monogenic diseases had been a long time challenge, especially for autosomal recessive disorders. The difficulty is caused by the coexistence of fetal and maternal DNA in maternal plasma, this makes difficult to distinguish the maternally inherited fetal allele from the background maternal DNA.

2 NIPT for single gene disorders

Relative Haplotype Dosage (RHDO) Analysis

The NIPT for single gene disorders combine the power of the targeted massive parallel sequencing (MPS) with a relative haplotype dosage (RHDO) test since it allows establishing both parental inheritances.

Taking an hypothetical typical autosomal recessive case where both parents are heterozygous carriers and the couple is expecting a second child, the RHDO process is carried out for a series of SNPs within the same genomic region in order to establish the inheritance from the fetus [Figure 2]. If the mother is a heterozygous carrier, the combinations of SNPs would therefore form 2 different haplotypes. One haplotype is named haplotype I and the other haplotype II respectively (HapI or HapII) [Figure 3]. In order to be informative, those SNPs positions need to be homozygous in the father. Those SNPs are classified into type α or type β. A type α SNP is one which the allele on the maternal haplotype is identical to the paternal allele at the same SNP position. In contrast, a type β SNP is the one which the allele on the maternal haplotype is different from the paternal one.

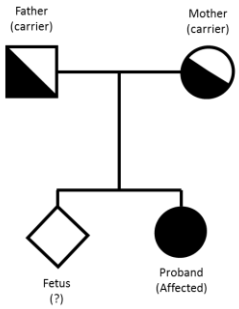

Figure 2. Schematic representation of a typical pedigree from an autosomal recessive case.

After that the RHDO test is performed and depending on which type of SNPs are overrepresented in the maternal plasma DNA it is possible to known which maternal haplotype has been inherited by the fetus [Figure 3]. Also, knowing which is the maternal mutant haplotype thanks to the proband, it is possible to establish if the fetus has inherited or not the causative mutation from the mother. The same process is applied for detect the paternal inheritance but in this case there is no need to perform a RHDO test because the detection of a paternal haplotype absent in the mother but present at the maternal plasma DNA will inform which paternal allele has been inherited by the fetus.

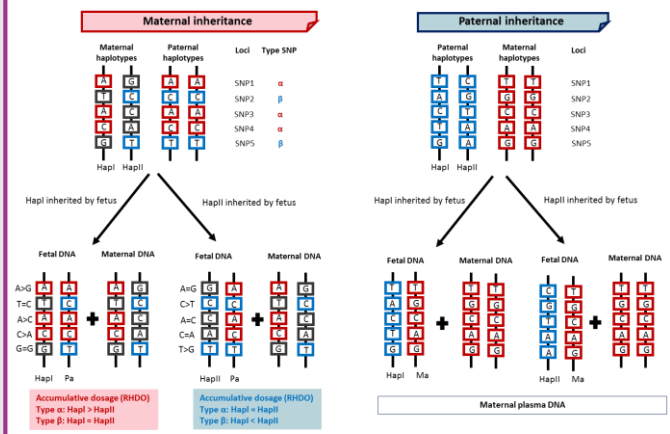

Figure 3. Relative Haplotype Dosage (RHDO) test for an autosomal recessive disease case where both parents are heterozygous. Pa (paternal haplotypes) and Ma (maternal haplotypes).

This is the common approach for NIPT and it could be applied to any type of monogenic diseases. A good example of the use that could have the clinical application of NIPT can be seen in the congenital adrenal hyperplasia (CAH). CAH is a recessive autosomal disease in which the excess of fetal androgen production causes genital virilization in female fetuses. The genital organogenesis begins at approximately 9 weeks of gestation but the invasive prenatal testing is performed as soon at 14 weeks of gestation making the preventive treatment with dexamethasone impossible. So if NIPT could be applied on this type of cases, the status of the fetus as affected or not could be established on time and the treatment could be applied avoiding the disease effects.

3 State of the art

New developments in NIPT

Despite the RHDO strategy works pretty well, the new strategies are focusing on guarantee higher resolution and a precise quantification of mutant fetal alleles circulating. This could be achieved by using the new single-molecule amplification and sequencing methodologies [Figure 4]. But despite all the research and the improvements in the field there are still today some challenges to overcome [Table 1].

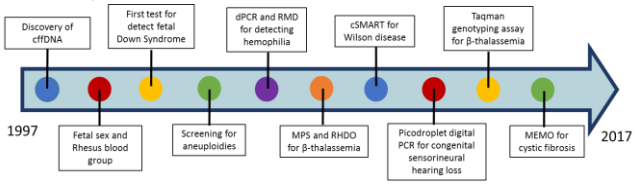

Figure 4. Schematic timeline that represents the advances in NIPT field since the discovery of cffDNA in 1997. The challenge is currently to tune up the protocols for NIPT employing the new single-molecule amplification and sequencing methodologies like cSMART (circulating single-molecule amplification and resequencing technology); picodroplet digital PCR; Taqman probes or MEMO (3'-Modified Oligonucleotide PCR). dPCR (digital PCR); RMD (relative mutation dosage) test.

Table 1. Current challenges for NIPT

cffDNA fraction is critical to the NIPT process	2.0% of NIPT error cases are caused by an insufficient amount of cffDNA. To avoid this type of error they are also working to improve the methods of quantification of the fraction of cffDNA in maternal plasma.
Mosaicism in the placenta	The mosaicism could affect the reliability of NIPT because of the mixture of DNA. This could be avoided by improving the reliability of the sequencing technologies allowing to distinguish between alleles.
Twin fetus in pregnancy	Especially on dizygotic twin pregnancies where it might be more difficult to determine which fetus harbors a mutation, unless the twins are of different sexes. Also it is needed more reliability on the sequencing technologies.
Discordance between the result of the NIPT and the reality	Many studies reported that some NIPT results were false positives or false negatives that were revealed through a posterior invasive prenatal diagnosis. More reliability is needed in order to avoid this problem.

CONCLUSIONS

First of all, the NIPT of recessive monogenic diseases constitutes just a complementary screening for invasive prenatal testing in clinical practice because of the still presents challenges. However, even if the definitive clinical application of the NIPT, at least as screening, is no longer a distant future, if at the end NIPT for autosomal recessive disorders and monogenic diseases in general could not be applied into clinical practice as an alternative for invasive prenatal testing it will not be because the technical challenges cannot be overcome. In our opinion, the cause will be all the social and ethical considerations which are equal, or even more important, than the reliability of the technique for the patients.

Relevant References
[1] Lo Y. M. D. et al. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. Sci. Transl. Med. (2010).
[2] Tounta, Georgia, et al. "Non-invasive prenatal diagnosis using cell-free fetal nucleic acids in maternal plasma: Progress overview beyond predictive and personalized diagnosis." EPMA Journal 2.2 (2011): 163-171.
[3] New, Maria I., et al. "Noninvasive prenatal diagnosis of congenital adrenal hyperplasia using cell-free fetal DNA in maternal plasma." The Journal of Clinical Endocrinology & Metabolism 99.6 (2014): E1022-E1030.
[4] Liu, Li, et al. "A Forward Look At Noninvasive Prenatal Testing." Trends in Molecular Medicine 22.11 (2016): 958-968
[5] Guissart, C., et al. "Non-invasive prenatal diagnosis (NIPD) of cystic fibrosis: an optimized protocol using MEMO fluorescent PCR to detect the p. Phe508del mutation." Journal of Cystic Fibrosis 16.2 (2017): 198-206.

UAB Universitat Autònoma de Barcelona